

ABSTRACT

THESIS: Vault RNA1 regulation of apoptosis in multidrug-resistant GLC4 small cell lung cancer cells

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Small cell lung cancer (SCLC) is an aggressive form of lung cancer that frequently develops multidrug resistance (MDR) during chemotherapy. Vault RNA1 (vRNA1), a non-structural component of the MDR-associated vault organelle, is believed to act as a microRNA (miRNA) and may contribute to MDR by regulating the expression of genes involved in apoptosis, inflammation, and/or drug metabolism. Since vaults function to aid cells in survival, we hypothesized that vRNA1 might be free in the cytoplasm and able to inhibit expression of pro-survival mRNAs when vaults are open in drug-sensitive GLC4/S cells but not in the MDR GLC4/ADR cells where vaults might be closed with the miRNA sequestered within. In order to establish the role of vRNA1 as a regulator of survival in SCLC cells, siRNA-mediated down-regulation of vRNA1 was employed in GLC4/S and GLC4/ADR SCLC cells.

Fluorescence microscopy using a green fluorescent 3' AlexaFluor-488 negative siRNA control was used to estimate transfection efficiency, yielding 56% for GLC4/S and 89% for GLC4/ADR. However, these values and the level of apoptosis before and after transfection, as judged by trypan blue hemacytometer cell counts, were not entirely reliable due to cell clumping. The latter counts indicated a 2-fold decrease in viability in GLC4/S cell following transfection but no decrease in GLC4/ADR cells ($p \leq 0.05$). RT-PCR revealed that transfection significantly ($p \leq 0.05$) decreased vRNA1 expression in GLC4/S cells but not in GLC4/ADR cells, confirming our hypothesis concerning the availability of vRNA1 in the two cell types. Caspase activity measurements showed vRNA1 down-regulation in the GLC4/ADR cells significantly ($p \leq 0.05$) increased survival via a 6.1-fold reduction in caspase 3/7 activity, further supporting our hypothesis. However, GLC4/S cells showed a similar loss of apoptosis when transfected with either sivRNA1 or the negative control siRNA. vRNA1 down-regulation did not significantly ($p \leq 0.05$) affect the expression of major pro-survival (Bcl-2, Bcl-xL), pro-apoptotic (Bad), or pro-inflammatory (IL-6, NF κ B p65) factors in either GLC4/S or GLC4/ADR cells. However, the drug metabolism protein CYP3A (previously shown by Persson et al., 2009 to be regulated by vRNA1) was significantly ($p \leq 0.05$) lowered (~16%) following vRNA1 down-regulation in the GLC4/S cells. In conclusion, we were successful in down-regulating vRNA1 which enhanced cell survival as hypothesized, but we were not able to identify new proteins regulated by vRNA1.